

## Genotyping protocol

EMMA ID: EM:10425

Gene symbol: Col13a1

Strain designation: FVB.129S6(B6)-Col13a1<sup>tm2Pih</sup>/Oulu

### Primers:

Primer name	Primer sequence (5'- 3')
EM09884 for	GCTGTAGCTTCATGAGCGCCTGA
EM09884 wtrev	CCTGACCAAGATTGCCGATCTGA
EM09884 mutrev2	CGCTTCTGGTGCCGAAA

### Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	EM09884 for	EM09884 wtrev	167
Mutant	EM09884 for	EM09884 mutrev2	290

### Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	2.0
Primer 1 (10 µM)	1.0
Primer 2 (10 µM)	1.0
10x Buffer (15 mM MgCl <sub>2</sub> )	3.0
MgCl <sub>2</sub> (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H <sub>2</sub> O	21.65
<b>Total</b>	<b>30.0</b>

### PCR program:

Time	Temperature (+ °C)	Cycles
5 min	95	
1 min	94	35
1 min	54	
1 min	72	
10 min	72	
∞	10	